

## EXAMPLE 8

### D-RTR Tetramer Inhibition of N-acetyl-PGP or N-methyl-PGP Induced PMN Polarization

5           The RTR complementary peptide has been shown to inhibit the polarization of polymorphonuclear leukocytes activated by N-acetyl-PGP. The complementary sequence, RTR, was designed to specifically interact hydrophatically with the PGP sequence in N-acetyl-PGP and, therefore, should also interact with the same  
10 sequence in N-methyl-PGP. The D-RTR tetrameric peptide was designed to inhibit N-acetyl-PGP or N-methyl-PGP induced polymorphonuclear leukocyte polarization, but have a greater  
stability *in vivo* by resisting proteolytic degradation.

A preliminary study showed that the D-RTR tetramer  
15 inhibited (mean  $\pm$  SD) 800  $\mu$ M N-acetyl-PGP induced polymorphonuclear leukocyte polarization as follows: 100 nM D-RTR tetramer = 37%  $\pm$  35% inhibition (n=7), 1  $\mu$ M D-RTR tetramer = 65%  $\pm$  26% inhibition (n=6) and 10  $\mu$ M D-RTR tetramer = 92%  $\pm$  6% inhibition (n=6). The D-RTR tetramer inhibited (mean  $\pm$  SD) 1 mM N-

methyl-PGP induced polymorphonuclear leukocyte polarization as follows: 1-10  $\mu$ M D-RTR tetramer =  $14\% \pm 10\%$  inhibition (n=5), 40-100  $\mu$ M D-RTR tetramer =  $45\% \pm 7\%$  inhibition (n=2) and 200-800  $\mu$ M D-RTR tetramer = 100% inhibition (n=5).

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### EXAMPLE 9

#### Results

All four complementary (antisense) peptides, containing the RTR sequence, showed substantial inhibition of N-acetyl-PGP activated polymorphonuclear leukocyte polarization (Table 1). The RTR tetrameric peptide was a powerful inhibitor of N-acetyl-PGP (ID<sub>50</sub> of 200 nM). The RTR dimer was much less potent (ID<sub>50</sub> of 105  $\mu$ M). Both monomers, RTR (ID<sub>50</sub> of 2.5 mM) and RTRGG (ID<sub>50</sub> of 2.1 mM), were only antagonistic at millimolar concentrations. Preincubation of the RTR tetrameric peptide with N-acetyl-PGP or neutrophils for 5 min did not change the results described above. An additional antisense peptide, ASA tetramer, failed to show any inhibition of polymorphonuclear leukocytes activated by N-acetyl-PGP.

**TABLE I**

Complementary Peptide Inhibition of N-acetyl-PGP Activated PMN

Polarization

Complementary Peptides	Antagonist Activity (ID <sub>50</sub> )	p-value
RTR tetramer	200 nM ± 75 nM	<0.001
RTR dimer	105 µM ± 68 µM	0.001
RTR monomer	2.5 mM ± 1.2 mM	<0.001
RTRGG monomer	2.1 mM ± 0.8 mM	<0.001
ASA tetramer	None, ≤ 4 mM	-----

5 \* Untreated PMNs (negative control) produced a polarization response of 7.8% ± 4.4% (n = 41). PMNs activated with 500 µM N-acetyl-PGP (positive control) produced a polarization response of 56.5% ± 16.4% (n = 41). This chemoattractant concentration was selected from the linear portion of the dose response curve, yielding

10 approximately 50% polarization after subtraction of the negative control values. Antagonistic activity (ID<sub>50</sub>, mean ± standard deviation) was interpolated from five dose response curves for each complementary peptide.